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The Effects of Angiotensin Converting Enzyme inhibitors (ACEI) on Human N-acetylseryl-aspartyl-lysyl-proline (AcSDKP) Levels: A Systematic Review

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DECLARATION

I, Ayanda Trevor Mnguni, hereby declare that the work on which this dissertation is based, is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, or is to submitted for another degree in this or any other university.

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SUMMARY

Tuberculous pericardial effusion is complicated by pericardial fibrosis which manifests with the clinical feature of constrictive pericarditis in 4.4 to 7.8% and constriction in at least 50% of cases (Mayosi et al., 2014). N-acetyl-seryl-aspartyl-lysylproline (AcSDKP) is a ubiquitous tetrapeptide with important antifibrotic properties that has recently been shown to be low in tuberculous pericardial effusion(Ntsekhe et al., 2012a). It has been postulated that the low levels of Ac-SDKP in tuberculous pericarditis may be the pathophysiological basis of the high rate of constriction. Angiotensin converting enzyme inhibitors (ACEI) increase AcSDKP levels in animal models with antifibrotic effects. ACEIs are therefore, candidate drugs for prevention of fibrosis in tuberculous pericarditis, if they can be shown to increase the levels of AcSDKP in human tissues.

Background

The genus Mycobacterium contains more than 140 species (Van Ingen, 2013) which are separated in three major groups, that is, M. tuberculosis, M. leprae, and mycobacteria other than M.tuberculosis and M. leprae, collectively referred to as non-tuberculous mycobacteria. Mycobacterium tuberculosis is an obligate human pathogen and the causative agent of tuberculosis (TB), which remains one of the leading global public health problems. According to the world health organisation's 2014 global report on tuberculosis, Tuberculosis (TB) still remains a major global health problem, responsible for ill-health among millions of people each year. TB ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV).The latest estimates included in the report state that there were 9.0 million new TB cases in 2013 and 1.5 million TB deaths (1.1 million among HIV-negative people and 0.4 million among HIV-positive people).The totals included are higher than those included in the 2013 global TB report, primarily because of upward revisions to estimates of the number of TB cases and deaths in Nigeria following the finalization of results from the country's first-ever national TB prevalence survey which was completed in 2012.

Anatomy of the Pericardium

The pericardium is a double-walled fibroserous sac that encloses the heart and the roots of the great vessels. It consists of the fibrous pericardium which is bound to the central tendon of the diaphragm by the pericardiacophrenic ligament. Anteriorly the fibrous pericardium is bound to the sternum by the sternopericardium ligament and posteriorly, it's bound by loose connective tissue structures in the posterior mediastinum. This arrangement ensures that the heart is tethered in place inside the fibrinous sac. The internal surface of the fibrinous pericardium is lined with a serous membrane which is referred to as the parietal layer of serous pericardium. It is this serous layer that is then reflected onto the heart at the great vessels (aorta, pulmonary trunk, and veins and venae cavae) and is referred to as the visceral pericardium. The pericardial cavity is the potential space between the opposing layers of the parietal and visceral layers of the serous pericardium. This space normally contains about 10-20ml of fluid which is there to enable the heart to move and beat in a frictionless environment. The parietal layer of the serous pericardium fuses to the internal surface of the fibrous pericardium.

The visceral layer of the pericardium forms the epicardium, which is the external layer of the heart wall and reflects from the heart and great vessels to become continuous with the parietal layer of serous pericardium whereby:

a) The aorta and pulmonary trunk leave the heart; a digit can be inserted into the transverse pericardial sinus located posterior to these large vessels and anterior to the superior vena cava.
b) The superior vena cava, the inferior vena cava and the pulmonary veins enter the heart; these great vessels are partly covered by the serous pericardium which forms the oblique pericardial sinus.

The arterial supply of the pericardium is mainly from a slender branch of the internal thoracic artery, the pericardiacophrenic artery, which often accompanies or at least parallels the phrenic nerve to the diaphragm. Smaller contributions of blood arise from the:

a) Musculophrenic artery-a terminal branch of the internal thoracic artery
b) Bronchial, esophageal and superior phrenic arteries-branches of the thoracic aorta
c) Coronary arteries (visceral layer of serous pericardium only).

The venous drainage of the pericardium is from the:

a) Pericardiacophrenic veins, tributaries of the brachiocephalic (or internal thoracic) veins
b) Variable tributaries of the azygous venous system.

The nerve supply of the pericardium is from the

a) Phrenic nerves (C3-C5)-primary source of sensory fibres. Pain sensations conveyed by the phrenic nerves are commonly referred to the skin of the ipsilateral supraclavicular region (top of shoulder on the same side)
b) Vagus nerve-function of which is uncertain
c) Sympathetic trunks-vasomotor

Tuberculous Pericarditis

The cardiac manifestations of tuberculosis include the development of tuberculosis pericarditis. According to the heart of Soweto study, Tuberculosis (TB) pericarditis was the second most common cause of cardiovascular morbidity and mortality after HIV-associated cardiomyopathy (Sliwa et al., 2012). It has been established that TB pericarditis can present in one of three forms, namely; pericardial effusions, effusive-constrictive pericarditis and constrictive pericarditis. With regards to the pathogenesis of TB pericarditis, the pericardium can get infected as a result of haematogenous spread from primary TB infection or retrograde lymphatic

spread of Mycobacterium TB from lymph nodes located in the tracheal, peribronchial and mediastinal regions, thereafter protein antigens form the TB bacillus induce delayed (type IV) hypersensitivity responses which in-turn stimulate the release of lymphocytes which in turn release lymphokines leading to the activation of macrophages and subsequent granuloma formation.

There have been 4 pathological phases which have been identified in TB pericarditis, namely:

a) Fibrinous exudation with a polymorphonuclear leukocytosis, associated with relatively abundant mycobacteria, and early granuloma formation with loose organization of macrophages and T cell

b) Serosanguineous effusion with a predominant lymphocytic exudate with monocytes and foam cells

c) Absorption of effusion with organization of granulomatous caseation and pericardial thickening caused by fibrin, collagenosis, and ultimately, fibrosis

d) Constrictive scarring: whereby the fibrosing visceral and parietal pericardium encase the heart in a fibrocalcific skin which impedes filling during diastole leading to the syndrome constrictive pericarditis.

Diagnosis

The symptoms and signs are usually non-specific and vague in TB pericarditis; a chest radiograph reveals an enlarged cardiac shadow in over 90% of cases and may reveal features of active tuberculosis and the presence or absence of pleural effusions. The Electrocardiographic (ECG) changes are virtually abnormal and range from non-specific ST-T changes, PR segment depression and ST elevation (in acute pericarditis), microvoltage (complexes<5mm in limb leads&<10mm in precordial leads) in pericardial effusions.

Atrial fibrillation has been shown to have occurred in 25% of patients with tuberculous pericardial effusions (Syed et al., 2012).The echocardiogram may reveal features suggestive of an exudative effusion but not specific for a tuberculous aetiology namely: frond like projections and thick ‘porridge-like’ fluid. Imaging in the form of CT/MRI scan of the chest where available

may be of assistance in diagnosing pericardial effusions and features suggestive of tuberculous effusion include a pericardial effusion and thickening >3mm associated with typical mediastinal and tracheobronchial lymphadenopathy (>10mm, hypodense centres, matting) with sparing of the hilar nodes.

Pericardiocentesis

Pericardiocentesis is mandatory for patients who have clinical features of cardiac tamponade - not only is it a therapeutic intervention, but it also assists in diagnosis. Diagnostic pericardiocentesis should be considered in all patients with suspected TB pericarditis. Pericardial fluid should be sent for biochemical tests to distinguish between exudate and transudate, cytology, white cell count and culture-which still remains the goal standard for diagnosis of tuberculosis, however tuberculous pericardial fluid has been shown to be paucibacillary with estimated culture and microscope smear based diagnostic accuracy of approximately 50% and 5% respectively and the 'turn-around' time of 4-6weeks is associated with a delay in diagnosis(Theron et al., 2014) (Reuter, Burgess, van Vuuren, & Doubell, 2006).

The Xpert MTB/Rif which is a quantitative polymerase chain reaction (PCR) and can assist in the rapid diagnosis of Mycobacterium Tuberculosis and rifampicin resistance, has been endorsed by the WHO for the diagnosis of pulmonary TB using sputum samples and validation studies using culture positive sputum samples from pulmonary TB patients showed a pooled sensitivity of 98% and 68% in smear-positive and smear negative cases ("Cochrane database for sytematic reviews," 2013). Indirect tests such as:

a) Adenosine deaminase (ADA) level which is the current available surrogate marker that suggests MTB infection, according to the South African national health laboratory services reference ranges for normal ADA levels are : 0-15U/L for serum,0-30U/L for pleural fluid and 0-9U/L for cerebrospinal fluid. Locally available, yet unvalidated data regarding ADA measurements in pericardial fluid suggests an ADA cut-off value of 40 U/L resulted in a test sensitivity, specificity, positive predictive value, negative predictive value and diagnostic efficiency of 84%, 80%, 91%, 66%, and 83%, respectively(Reuter, H, Burgess, LJ, Carstens, ME, Doubell, 2005)

b) Unstimulated Interferon Gamma assay-Pandie et al assessed the diagnostic utility of Xpert MTB/RIF test compared to ADA and unstimulated interferon gamma assay in the

diagnosis of Tuberculous pericardial fluid in a population with a high disease burden of TB and he was able to show that unstimulated interferon gamma offered superior accuracy for the diagnosis of microbiologically confirmed TBP compared to the new Xpert MTB/RIF test and the established ADA assay. Unstimulated interferon gamma assay maybe the optimal first line test for the diagnosis of TB pericarditis (Pandie et al., 2014)

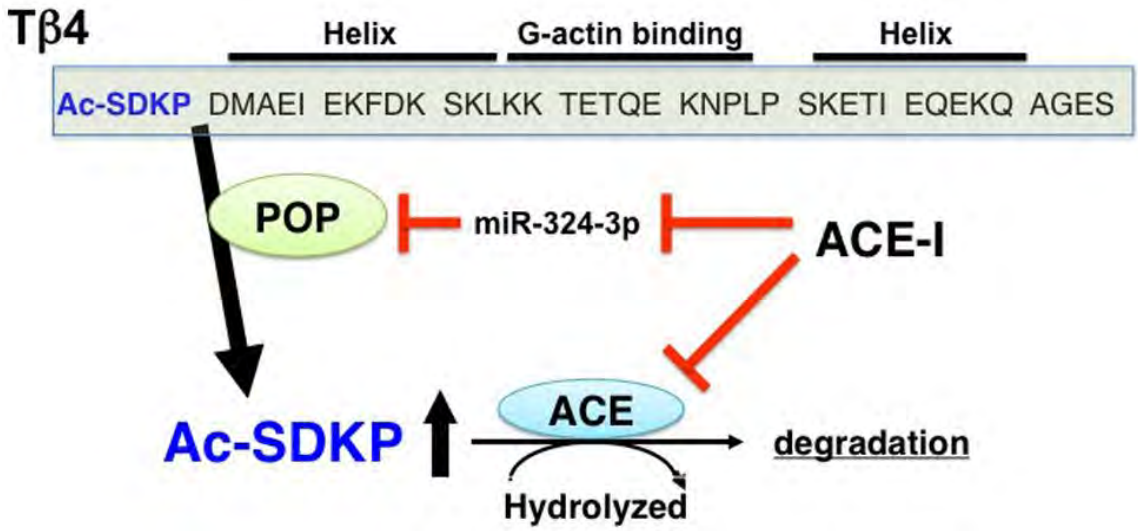
Management

Antituberculosis chemotherapy increases survival dramatically in tuberculous pericarditis. In the pre-antibiotic era, mortality was 80% to 90% (Harvey & Whitehill, 1937). A regimen consisting of rifampicin, isoniazid, pyrazinamide, and ethambutol for at least 2 months, followed by isoniazid and rifampicin (total of 6 months of therapy) has been shown to be highly effective in treating patients with extrapulmonary TB (Cohn, DL, Catlin, BJ, Peterson, KL, Judson, FN, Sbarbaro, 1990). Treatment for 9 months or longer gives no better results and has the disadvantages of increased cost and poor compliance. The role of corticosteroids in the management of TB pericarditis has been addressed by the Investigation of the Management of TB pericarditis in Africa (IMPI) - a multicentred randomized controlled trial which evaluated the effects of adjunctive glucocorticoid therapy and mycobacterium indicus pranii immunotherapy in patients with tuberculous pericarditis (Mayosi et al., 2014). There was no significant difference in the primary outcome between patients who received prednisolone and those who received placebo.

The treatment of tuberculous pericardial constriction involves the use of standard antituberculosis drugs for 6 months and pericardiectomy for persistent constriction in the face of drug therapy. Mutyaba et al, investigated the causes of constrictive pericarditis, outcomes after pericardiectomy, and predictors of mortality in Cape Town, South Africa, during a 22-year period of high HIV/AIDS prevalence. He found that tuberculosis is the main cause of constrictive pericarditis in South Africa, and that despite its efficacy at relieving the symptoms of heart failure, pericardiectomy was associated with high perioperative mortality that was not influenced by HIV status and that the New York Heart Association functional(class IV) and hyponatremia where predictors for early mortality after pericardiectomy (Mutyaba et al., 2014)

Angiotensin Converting Enyme inhibitors (ACEI) and N-Acetyl-Seryl Aspartyl-Lysyl-Proline (AcSDKP)

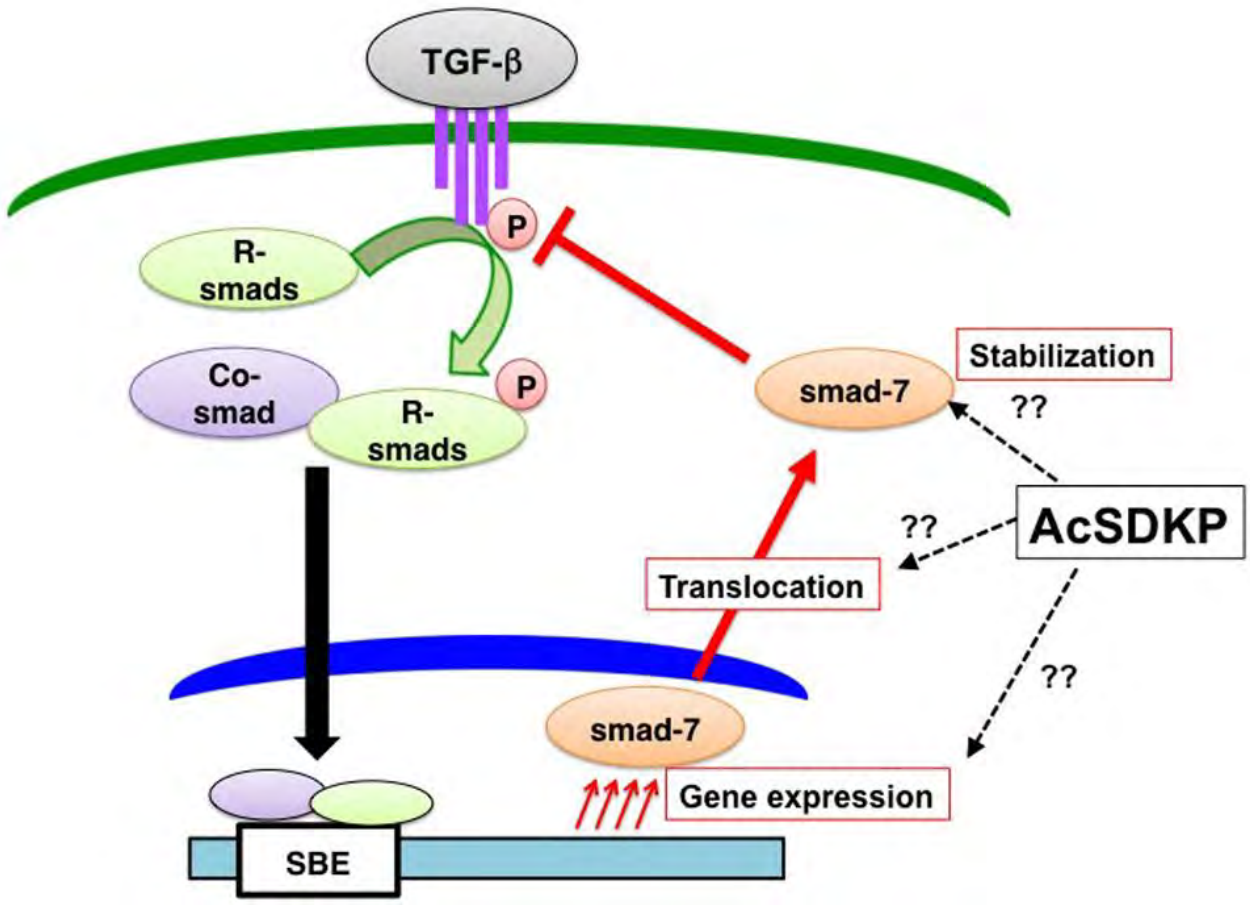
It is known that somatic angiotensin converting enzyme (ACE) consists of two homologous catalytically active domains (designated N- and C- domains) that share high overall sequence identity and structural topology (Bernstein et al., 2011; Wei, Alhenc-Gelas, Corvol, & Clauser, 1991). Despite the high degree of similarity between domains, each domain displays differences in substrate processing and inhibitor binding abilities. Bradykinin is hydrolyzed at approximately the same rate by both these catalytic sites (Bernstein et al., 2011; Wei et al., 1991). ACE has a range of substrates, including N-acetylseryl-aspartyl-lysyl-proline (AcSDKP). AcSDKP is cleared almost exclusively by ACE and specifically by the N-domain active site of this enzyme (Rousseau, Michaud, Chauvet, Lenfant, & Corvol, 1995). The details of the synthetic pathways of endogenous AcSDKP still remain unclear but the information available strongly suggests that thymosin β4 (Tβ4) is responsible. Thymosin β4 (Tβ4), which is a precursor to AcSDKP, is degraded by prolyl oligopeptidase, a ubiquitously distributed 43-amino-acid (4,9kDa),that was originally identified as an intracellular peptide, which can sequester G-actin and regulate its polymerization (Grillon et al., 1990).



Thymosin β4, a G-actin binding peptide, is cleaved by POP and subsequently its N-terminal tetrapeptide, AcSDKP, is synthesized. AcSDKP is hydrolyzed and degraded by ACE. ACE-I may suppress miR-324-3p, which may inhibit protein expression of POP. Therefore, the mechanisms underlying the increased levels of AcSDKP by ACE-I may include both the suppression of degradation pathway and the induction of synthesis pathway of AcSDKP

***FIGURE 1**: Synthesis and metabolism of AcSDKP*

AcSDKP is a tetrapeptide that was originally isolated from fetal calf bone marrow and has emerged as an antifibrotic molecule. AcSDKP was originally identified as a hematopoietic stem cell regulator and inhibits cell cycle progression stimulated by serum derived or platelet-derived growth factor β in human mesangial cells by inhibiting the degradation of p53,p27,and p21 (Kanasaki et al., 2006). AcSDKP further inhibits apoptosis induced by cytotoxic stresses, including chemotherapy (Bogden et al., 1991; Grillon et al., 1993), radiation (Deeg et al., 1997; Watanabe et al., 1996), high temperature (P. Wierenga, Brenner, & Konings, 1998; P. K. Wierenga, Setroikromo, Vellenga, & Kampinga, 2000; P. Wierenga & Konings, 1994), and photofrin -II-mediated phototherapy (Coutton, Guigon, Bohbot, Ferrani, & Oberling, 1994). Increased apoptosis is associated with tissue fibrosis and its inhibition has been linked to the restoration of fibrosis in several organs (Coward, Saini, & Jenkins, 2010; Dooley, Harvey, & Thomas, 2011; Gieling, Burt, & Mann, 2008; Rodriguez-Iturbe & Garcia Garcia, 2010). Inflammation is also associated with tissue fibrosis (Coward et al., 2010; Dooley et al., 2011; Gieling et al., 2008; Rodriguez-Iturbe & Garcia Garcia, 2010). AcSDKP mediates its antifibrotic effects by blunting the effects of TGFβ signalling through down regulation of the TGFβ/small mothers-against decaplentaplegic (Smad 2) and signal-regulated kinase (ERK 1/2) pathways (Border & Noble, 1994; Kanasaki, Koya, Sugimoto, & Isono, 2003; Miyazo, 2000; Pokharel et al., 2002). Effects are mediated by galectin-3 (Liu et al., 2009; Ntsekhe et al., 2012b), a TGFβ releasing macrophage recruiting and cardiac dysfunction molecule, are known to be blunted by this mechanism. AcSDKP reverses inflammation and fibrosis in rats with heart failure after myocardial infarction(Yang et al., 2004).The plasma level of AcSDKP in humans has been shown to increase by fivefold after acute administration of the ACE-inhibitor Captopril (Azizi et al., 1996).



Once TGF-β binds to TGF-β receptors on the cell membrane, the TGF-β and TGF-β-receptor interaction induces phosphorylation of receptor-regulated(R)-Smads. Phosphorylated R Smads interact with the common (co)-Smad in the cytoplasm. These Smad heterodimers in the nucleus then bind to the genomic promoter region of DNA, which is called the Smad-binding element (SBE). AcSDKP may induce Smad7 gene expression, protein stabilization, or translocation from the nucleus of cells to the cytoplasm as well as inhibit phosphorylation of R-Smads by TGF-β receptors. However, detailed mechanisms are not yet known.

FIGURE 2: AcSDKP is an anti-TGF-β/Smad peptide

Conclusion

The incidence of TB pericarditis in sub-Saharan Africa continues to increase as the result of the human immunodeficiency virus (HIV) epidemic (Cegielski, Lallinger, Ramaiya, Mtulia, & Mbanga, 1990). Constrictive pericarditis is one of the most devastating complications of TB pericarditis occurring in 4-8% of patients despite prompt antituberculous treatment and corticosteroids, and is associated with significant morbidity and mortality (Mutyaba et al., 2014). TB pericarditis is associated with decreased levels of pericardial AcSDKP (Ntsekhe et al., 2012b); ACE-i via their ability to elevate AcSDKP levels are therefore candidates for the treatment of tuberculous pericarditis to prevent fibrotic constriction which would greatly improve the mortality and enable clinicians to improve the quality of life of the affected patients with pharmacological agents which are cheap, easily available and thus abolish the need for pericardiectomy.

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PART B: Journal Ready Manuscript

The Effects of Angiotensin Converting Enzyme inhibitors (ACEI) on Human N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) Levels: A Systematic Review and Meta-Analysis

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Abstract

***Background*:** Tuberculous pericardial effusion is a pro-fibrotic condition that is complicated by constrictive pericarditis in 4-8% of cases. N-acetyl-seryl-aspartyl-lysylproline (Ac-SDKP) is a ubiquitous tetrapeptide with antifibrotic properties that is low in tuberculous pericardial effusion, thus providing a potential mechanism for the heightened fibrotic state. Angiotensin converting enzyme inhibitors (ACEI), which increase Ac-SDKP levels with antifibrotic effects in animal models, are candidate drugs for preventing constrictive pericarditis if they can be shown to have similar effects on AcSDKP and fibrosis in human tissues.

Objective: To systematically review the effects of ACEIs on Ac-SDKP levels in human tissues.

Methods: We searched five electronic databases (1996-2014) and conference abstracts with no language restrictions. Two reviewers independently selected studies, extracted data and assessed methodological quality. The protocol was registered in PROSPERO.

Results: Four studies with a total of 206 participants met the inclusion criteria. Three studies (106 participants) assessed the change in plasma levels of AcSDKP following ACEI administration in healthy humans. The administration of an ACEI was associated with an increase in AcSDKP levels (mean difference (MD), 5.07 pmol/ml (95% confidence intervals (CI) 0.64 to 9.51 pmol/ml)). Two studies with 100 participants further assessed the change in AcSDKP level in humans with renal failure using ACEI. Administration of an ACEI was associated with a significant increase in AcSDKP levels (MD, 8.94 pmol/ml; 95% CI 2.55 to 15.33; I^2 = 44%).

Conclusion: ACEI increased AcSDKP levels in human plasma. These findings provide the rationale for the testing of the impact of ACEI in on AcSDKP levels and fibrosis in tuberculous pericarditis.

Introduction

The incidence of tuberculous (TB) pericarditis in sub-Saharan Africa is increasing as the result of the human immunodeficiency virus (HIV) epidemic (1)(2). TB pericarditis caused by *Mycobacterium tuberculosis* is found in 1% of all autopsied cases of TB and in 1-2% of instances of pulmonary TB (3). It is the most common cause of pericarditis in Africa and other developing countries in which TB remains a major public health problem (3). Constrictive pericarditis is one of the most devastating complications of TB pericarditis occurring in 4-8% of cases, despite prompt antituberculosis treatment and corticosteroids (4). The treatment of TB pericardial constriction involves the use of standard antituberculosis drugs for 6 months and pericardiectomy for persistent constriction in the face of drug therapy.

Mutyaba and others investigated the causes of constrictive pericarditis, outcomes after pericardiectomy, and predictors of mortality in Cape Town, South Africa, during a 22-year period of high HIV/AIDS prevalence. They found that TB is the main cause of constrictive pericarditis in South Africa, and that despite its efficacy at relieving the symptoms of heart failure, pericardiectomy was associated with high perioperative mortality of 16% that was not influenced by HIV status. New York Heart Association Functional Class IV and hyponatremia were predictors of early mortality after pericardiectomy (5).

TB pericarditis is associated with decreased levels of the anti-fibrotic agent N-acetylseryl-aspartyl-lysyl-proline (AcSDKP) levels (6), whereas ACEIs are known to increase AcSDKP levels in rodent tissues (7). AcSDKP is a potent antifibrotic agent and a negative regulator of hematopoietic stem cell differentiation. If ACEIs increase AcSDKP levels in human tissues, then they would be candidate drugs for use in TB pericarditis to prevent fibrosis and constriction (8)(9)(10)(11). We have conducted a systematic review of the literature to determine whether ACEI increase AcSDKP levels in human tissues.

Methods

These methods are based on our protocol which was registered in Prospero (12).

Elligibility Criteria

Types of participants

Only studies incorporating human participants will be considered.

Types of interventions

Interventions must include any ACE-I, whether alone or as part of other interventions.

Types of outcome measures

Effects of ACE-I on AcSDKP levels in body tissues.

Types of comparison or control interventions

Any placebo

Primary outcomes

Change in AcSDKP levels as detected by standardised laboratory assays / protocols following ACE-i administration in humans.

Secondary outcome

Effect of changes in AcSDKP level on fibrotic diseases.

Search Strategy

Two authors (ATM and MEE) undertook a systematic literature search of a number of databases for studies of the effects of ACEI on human AcSDKP levels. Potentially, relevant studies were selected on the basis of title and abstract for scrutiny without language restriction. The following databases where searched: PubMed, Google Scholar, EMBASE, and the Cochrane Library. A combination of the following search terms (including the use of MeSH) was used: angiotensin-converting enzyme, angiotensin-converting enzyme inhibitors, human, *N*-acetyl-seryl-aspartyl-lysyl-proline, AcSDKP. The search strategy is outlined in Table 1. The reference lists of identified articles were reviewed. Authors and experts undertaking research in the field of ACEI and AcSDKP were also consulted. Studies selected for review were prospective observational studies of the effects of ACEI on human AcSDKP levels.

Table 1. PUBMED SEARCH STRATEGY (*Adapted for use in other databases*)

#1	("angiotensin converting enzyme inhibitors" OR "ACE inhibitors")
#2	("N-acetylseryl-aspartyl-proline level" OR AcSDKP level)
#3	(#1 AND #2) Filters: Humans

Data Extraction and Management

Data were extracted by two authors (ATM and MEE) using a standardised data extraction form. Data were entered into Review Manager 5.1 statistical software for meta-analysis. Any disagreements on eligibility of articles for inclusion were discussed with BMM.

Quality Assessment

All articles included were critically appraised by two authors (ATM and MEE) for methodological quality in accordance with the methods of the Cochrane Collaboration (13). Each included article was assessed for risk of bias based on sequence generation, allocation concealment, blinding, and incomplete outcome or missing data, where applicable. Heterogeneity between studies was assessed using the chi-square test set at a 10% level of significance (13). The impact of any statistical heterogeneity was quantified using the I^2 statistic. If there was an acceptable degree of heterogeneity and it was appropriate to pool the data, the Mantel-Haenszel statistical method and Random Effects Analysis Model were used with the results presented in the form of a meta-analysis.

Data Synthesis and Analysis

Two authors (ATM and MB) reviewed all the relevant articles identified from the search, and after scanning the titles, identified those which were for potential inclusion, subject to reading the abstracts. The full text of the articles was obtained for final evaluation for inclusion into the review according to the pre-specified inclusion criteria. The PRISMA guideline was used in reporting the findings of this review (14). The outcome (i.e., effect of ACEI on AcSDKP level) was considered as a continuous variable. The outcome measure was calculated using risk ratios and 95% confidence intervals. Outcomes expressed in ng/ml were converted to pmol/ml by dividing the ng/ml value by the molecular weight of AcSDKP (487 Daltons) $X10^{-3}$. Interquartile ranges were converted to standard deviations as per recommended methods (Cochrane Handbook).

Results

Seventy-four papers where identified by electronic search of which 55 papers were excluded based on title and abstract (Fig. 1). A further 15 papers were excluded following full review of the text given that they were animal studies (n=8) or not related to primary outcome (n=7). Thus, four studies met with the inclusion criteria (Azizi 1996, Azizi 1997, Azizi 1999, Inoue 2010). The included studies were conducted in France (Azizi 1996, Azizi 1997, Azizi 1999) and Japan (Inoue 2010). The studies in France included healthy subjects (Azizi 1996), patients with hypertension (Azizi 1997) and patients with renal failure (Azizi 1999). The study in Japan (Inoue 2010) included a combination of healthy patients and those with renal failure. Included studies are described in Table 2. The reasons for excluding studies, initially considered relevant, are provided in Table 3.

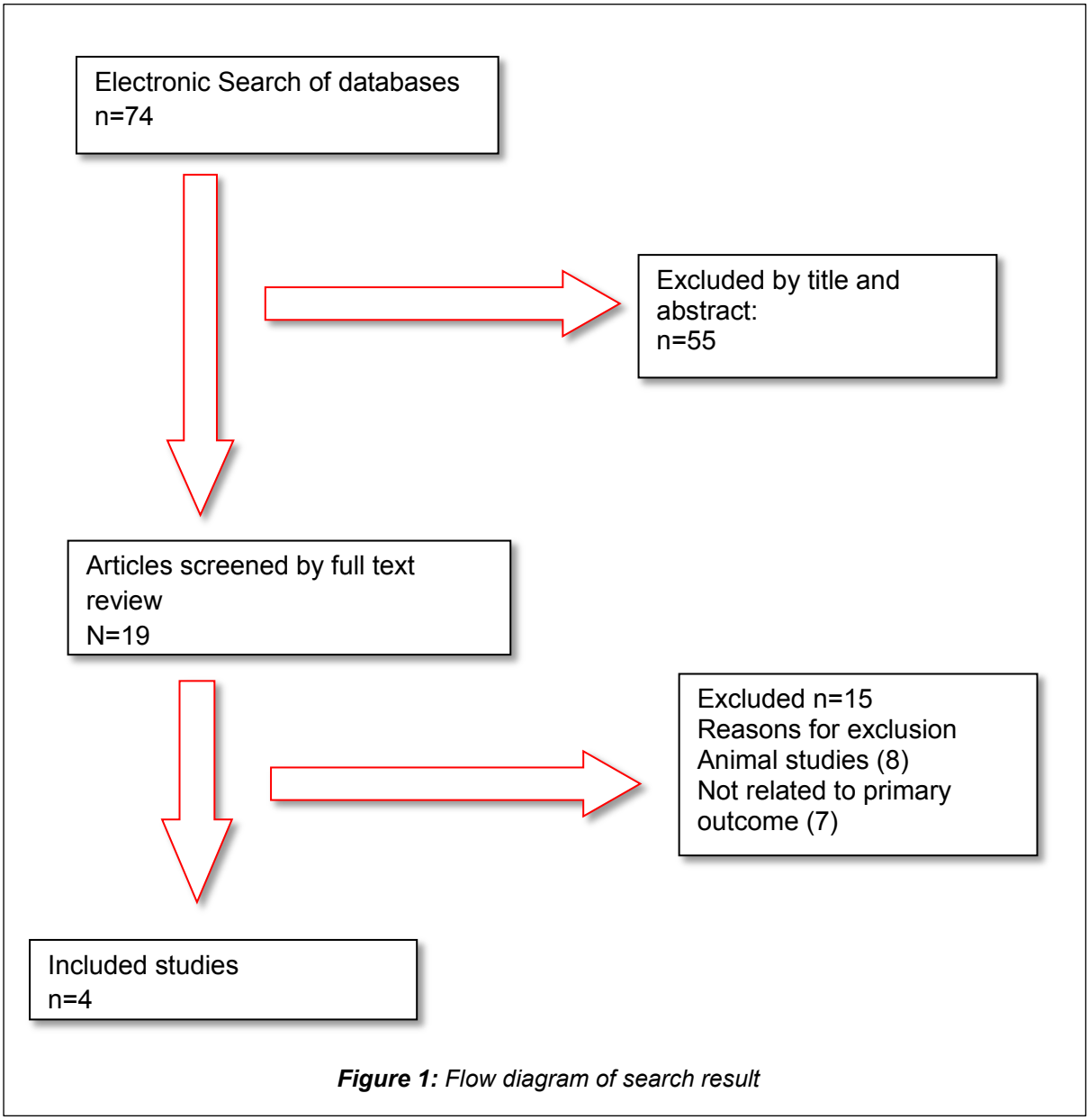

Figure 1: *Flow diagram of search result*

Table 2. Characteristic of studies included in the review

Study Id	Methods	Participants	Intervention/Control	Outcome
Azizi 1996	Single dose, double- blind two-way crossover study design set in Centre d' Investigations Cliniques, Hospital Broussais, Paris	16 Caucasian healthy male volunteers, age range : 20-35 years	Intervention: captopril (50mg) versus 50ml of water (n=8). Control = 8 patients received placebo and 50ml of water	Rise in AcSDKP level in blood following ACEI (Captopril)
Azizi 1997	Prospective cohort study set at Broussais Hospital, Paris	50 white hypertensive patients of both sexes aged : 18-75	Varying dosages of ACE-I were used; 27 patients (21: M, 6: F) on ACEI. Age range: 58+/-12 years, SBP: 164+/-33 mmHg. Control: 23 patients (17: M, 6:F) not on ACEI. Age range: 55+/-8 years; SBP:161+/-21 mmHg	Plasma AcSDKP levels elevated in patients on ACEI
Azizi 1999	Observational study set at Broussais Clinical Investigation Center	32 patients on the single oral dose; 12 patients on the multiple oral doses; 58 patients with CRF; 40 patients normal renal function	Single oral dose study: 32 patients - Captopril (50mg) with 25ml of water Multiple Oral Dose study:12 patients 10 : Captopril(50mg) with 25ml of water; 2: placebo with 25ml of water 58 patients: 35 on ACEI; 23 not on ACEI 40 patients with normal renal function:19 on ACEI; 21 not on ACEI	1. Renal failure was associated with a slight increase in plasma AcSDKP levels; 2. AcSDKP levels were increased in patients with normal renal function treated with an ACEI but only moderate because of ACE was intermittently reactivated between doses
Inoue 2010	Observational study set at Meiyo clinic Japan	41 patients: 7 healthy, 34 on dialysis. 28 dialysis patients: 10, Enalapril; 18, Trandolapril	Existing patients on ACEI – no dosages stated.	Study focused on relatively simple and highly sensitive and specific analytical method for the quantitative determination of AcSDKP and AcSDKP minor in human plasma samples using SPE and LC-MS/MS in the MRM mode

ACEI, angiotensin converting enzyme inhibitor; CRF, chronic renal failure; SBP, systolic blood pressure; LC-MS/MS, liquid chromatography- tandem mass spectrometry, SPE, solid phase extraction, MRM, multiple reaction monitoring, AcSDKP minor-synthesised from thymosin $\beta_{1\ 0}$

Table 3: Characteristics of Excluded Studies

Study ID	Reason for exclusion
(15) Bogden,1991	Animal study
(16) Cashman,1994	Animal study
(17) Comte,1998	Animal study
(18) Struthers,1999	Not related to primary outcome
(19) Azizi,2000	Not related to primary outcome
(20) Azizi,2001	Not related to primary outcome
(21) Peng,2003	Animal study
(7) Cavasin,2004	Not related to primary outcome
(22) Rasoul,2004	Animal study
(23) Azizi,2006	Units of measurement provided as a ratio (AcSDKP/Creatinine)
(24) Cavasin,2007	Animal study
(25) Lin,2013	Animal study
(26) Liu,2009	Not related to primary outcome
(27) Wang,2010	Animal study
(28) Nakagawa,2012	Not related to primary outcome

Change in AcSDKP levels in healthy participants

Three studies (106 participants) assessed the change in the levels of AcSDKP following ACE-I administration in healthy humans (9,10,29). Given the high statistical heterogeneity between studies (I^2 = 81%), a random-effects model was used. The administration of an ACE-I was associated with an increase in AcSDKP levels (mean difference (MD), 5.07 pmol/ml (95% confidence intervals (CI) 0.64 to 9.51 pmol/ml). (Fig. 2). After exclusion of the trial with a small number of participants (Azizi et al., 1996), the effect of ACE-I on AcSDKP levels remained significant with a mean difference of 2.62 pmol/ml (95% CI 0.93 to 4.31).

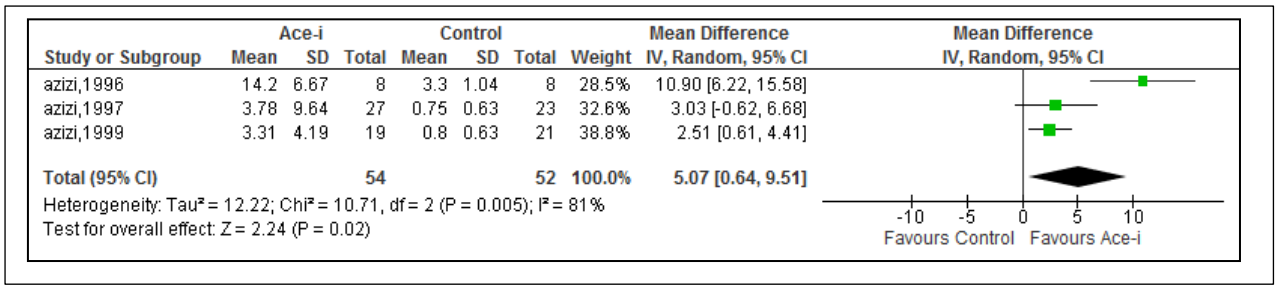

Figure 2: Change in AcSDKP levels in healthy participants. IV; inverse variance

Change in AcSDKP levels in participants with renal failure

Two studies with 100 participants assessed the change in AcSDKP level in humans with renal failure using ACE-I (29,30). One study administered Captopril (29), while the second (30) used two types of ACE-I namely enalapril and trandolapril. Administration of an ACE-I was associated with a significant increase in AcSDKP levels (MD, 8.94 pmol/ml; 95% CI 2.55 to 15.33; I^2 = 44%) (Fig.3).

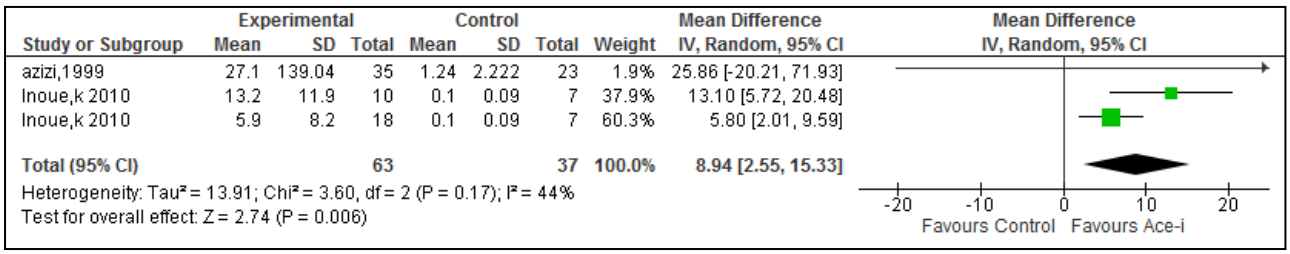

Figure 3: Change in AcSDKP levels in participants with renal failure

Methodological Quality

Table 3 shows the risk of bias assessment which included the components of random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete data and selective outcome reporting. All these components were assessed as being either low risk, high risk or unclear. There were no missing data in any of the studies.

Table 3: Risk of Bias Assessment

Study ID	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome Data	Selective Outcome Reporting
Azizi 1996	Low risk	Unclear	Low risk	Low risk	Low risk	Low risk
Azizi 1997	N/A	High risk	High risk	High risk	Low risk	Low risk
Azizi 1999	N/A	High risk	High risk	High risk	Low risk	Unclear
Inoue 2010	N/A	High risk	High risk	High risk	Unclear	Unclear

N/A; Not applicable

Discussion

This study shows that ACEI increase plasma levels of AcSDKP in humans. This effect is present in health and disease, and appears to be a class effect of ACEI. These findings are consistent with observations of animal models. There were, however, no studies of the impact of ACEI in other body tissues such as the pericardium, nor were there studies of the effect of higher levels of AcSDKP on tissue fibrosis.

The hypothesis that treatment with ACEI may increase the levels of AcSDKP in patients with TB pericarditis that was put forward by Ntsekhe and others is supported by this study (Ntsekhe et al 2012). Our findings open the way for experiments to determine whether ACEI can safely increase AcSDKP levels in pericardial fluid. However, the hypotensive effect of ACEI may be deleterious in patients with haemodynamic instability caused by TB pericarditis. The question of the safety of ACEI in TB pericarditis may be examined in prospective studies such as the IMPI trial where patients were treated for the clinical syndrome of heart failure(4).

The inclusion of four studies with a total of 206 participants from France and Japan may be seen as a limitation of this study. It is reassuring however that the direction of effect of ACEI on AcSDKP was consistent in this study, and followed the biological expectation. The findings therefore have both internal and external validity, and are likely to be of general relevance.

Conclusions

ACE inhibition elevates AcSDKP levels in human plasma. These findings provide the rationale for further studies being needed so as to allow for the investigation of the effect of ACEI on AcSDKP levels in pericardial fluid and impact on the incidence of constriction in tuberculous pericarditis.

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PART C: Appendices

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INCLUSION/EXCLUSION AND DATA EXTRACTION

The effects of ACE-i on human AcSDKP Levels

Study Information

First author:	Year:	Study ID:	Study title:

PART I: Eligibility

Study detail	Eligibility criteria *(Tick as appropriate)*	Eligibility criterion fulfilled *(Tick as appropriate)* Yes	No	Unclear	Location in text
Participant	☐ Human participants ☐ Animal models	☐	☐	☐	
Intervention	☐ ACE inhibitor	☐	☐	☐	
Comparator	☐ Standard of care ☐ Placebo ☐ Nothing	☐	☐	☐	

Outcome	☐ Change in serum AcSDKP level	☐ ☐ ☐	
Study design	☐ Observational ☐ Clinical controlled trial	☐ ☐ ☐	

Study eligibility *(tick as appropriate)*		
☐ INCLUDE	☐ EXCLUDE	☐ UNCLEAR
	Reason for exclusion:	*Reason for uncertainty:* *Contact author*

Author contact details:

DO NOT PROCEED IF EXCLUDED FROM REVIEW

PART II: Population and setting

	Description	Location in text
Study population description *(from which participants are drawn)*		
Setting *(location, social context)*		
Inclusion criteria		
Exclusion criteria		
Method/s of recruitment of participants		
Notes:		

PART III: Methods

	Descriptions as stated in report/paper	Location in text
Aim of study		
Design *(parallel, crossover, cluster)*		
Assessment Method		
Method of Randomisation		

Notes:

PART IV: Participants

	Intervention	Control	Total
Age range			
Sex			
Race/Ethnicity			
Severity of illness			
Co-morbidities			
Other treatment received *(additional to study intervention)*			
Notes:			

PART V: Treatment Arms

Group name	Intervention	Contorl	Total
No. randomised to group *(specify whether no. people or clusters)*			
Description Of Intervention *(include sufficient detail for replication, e.g. content, dose, components)*			
Duration of treatment period			
Timing *(e.g. frequency, duration of each episode)*			
Delivery *(e.g. mechanism, medium, intensity, fidelity)*			
Providers *(e.g. no., profession, training, ethnicity etc. if relevant)*			
Co-interventions			

Notes:

PART VI: Outcomes

Primary outcome: change in AcSDKP LEVELS following ACEi administration

	Description as stated in report/paper	Location in text
Outcome name		
Time points measured		
Time points reported		
Outcome definition *(with diagnostic criteria if relevant)*		
Person measuring/reporting		
Unit of measurement		
Scales: upper and lower limits *(indicate whether high or low score is good)*		
Is outcome/tool validated?	☐ Yes ☐ No ☐ Unclear	
Imputation of missing data *(e.g. assumptions made for ITT analysis)*		
Assumed risk estimate (*e.g. baseline or population risk noted in background)*		
Power		
Notes:		

Secondary outcome: EFFECT OF ACE-I ON BLOOD PRESSURE ON PARTICIPANTS

	Description as stated in report/paper	Location in text

Outcome name		
Time points measured		
Time points reported		
Outcome definition *(with diagnostic criteria if relevant)*		
Unit of measurement		
Is method of measurement validated?	☐ Yes ☐ No ☐ Unclear	
Notes:		

PART VII: RESULTS

PRIMARY OUTCOME RESULTS: CHANGE IN Acsdkp levels following acei administration

Intervention Control Total

Comparison							
Outcome							
Subgroup							
Results	**Intervention**			**Comparison**			
	µ	Variance	No. participants	µ	Variance	No. participants	
No. missing participants and reasons							
No. participants moved from other group and reasons							

Any other results reported		
Time Points measured		
Time points reported		
Is measurement method validated		
Adverse Events)		
Notes:		

	Description as stated in report/paper						**Location in text**
Comparison							
Outcome							
Subgroup							
Post-intervention or change from baseline?							
Results	**Intervention**			**Comparison**			
	µ	Variance	No. participants	µ	Variance	No. participants	
No. missing participants and reasons							
No. participants moved from other group and reasons							

Any other results reported		
Time points reported		
Time points measured		
Is measurement method validated		
Adverse events		
Notes:		

PART VIII: Risk of bias assessment

Domain	Risk of bias: Low risk	Risk of bias: High risk	Risk of bias: Unclear	Supporting evidence	Location in text
Random sequence generation *(selection bias)*	☐	☐	☐		
Allocation concealment *(selection bias)*	☐	☐	☐		
Blinding of participants and personnel *(performance bias)*	☐	☐	☐		
Blinding of outcome assessment *(detection bias)*	☐	☐	☐		
Incomplete outcome data *(attrition bias)*	☐	☐	☐		
Selective outcome reporting *(reporting bias)*	☐	☐	☐		
Notes:					

PART IX: Applicability

Have important populations been excluded from the study? *(consider disadvantaged populations, and possible differences in the intervention effect)*	☐ Yes ☐ No ☐ Unclear	**Notes:**
Is the intervention likely to be aimed at disadvantaged groups? *(e.g .lower socioeconomic groups)*	☐ Yes ☐ No ☐ Unclear	**Notes:**
Does the study directly address the review question? *(any issues of partial or indirect applicability)*	☐ Yes ☐ No ☐ Unclear	**Notes:**
Notes:		

PART X: Other information

	Description as stated in report/paper	**Location in text**
Key conclusions of study authors		
References to other relevant studies		
Correspondence required for further study information		
Notes:		